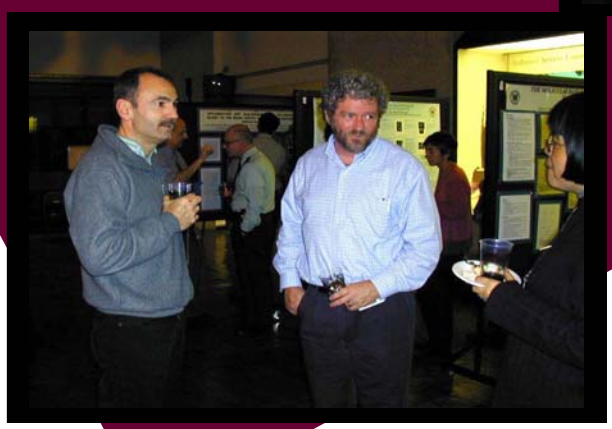
March 2003
Volume 10, Issue 2

USUHS Neuroscience Program
www.usuhs.mil/nes/home.html

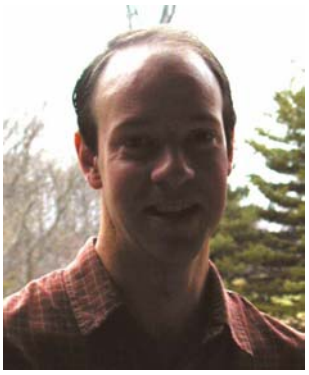
Neuroscience Newsletter

Neuroscience Open House Draws Crowd



Three New Faculty Join Neuroscience Program

Dr. J. Brian McCarthy received his BS in Biology from Juniata College, MS in Molecular Biology from University of Maryland, PhD in Neurobiology from State University of New York at Stony Brook, and postdoctoral training in the Department of Cellular and Molecular Physiology at the Yale University School of Medicine. Prior to joining USUHS as an Assistant Professor of Pharmacology in June 2002 , he held the position of Instructor in the Division of Neuroscience/ Department of Neurology at Cornell University Medical College. His laboratory investigates the cellular and molecular mechanisms that underlie synapse-specific modifications, including: synaptic receptor targeting, local protein synthesis, and the neuroendocrine regulation of local protein synthesis and synaptogenesis.



Dr. David J. Mears, an assistant professor in the Department of Anatomy, Physiology & Genetics, received his BS in Biomedical Engineering from Boston University, PhD in Biomedical Engineering from Johns Hopkins University and postdoctoral training in the Biophysics and Islet Physiology Sections, Laboratory of Cell Biochemistry and Biology at NIDDK, NIH. The goals of Dr. Mears' laboratory are to elucidate the cellular and molecular mechanisms involved in physiological regulation of insulin secretion and how these pathways are involved in the pathogenesis of diabetes. Insulin secretion from pancreatic β-cells is tightly regulated by plasma glucose levels, circulating hormones and locally released neurotransmitters. Defects in the responsiveness of the β-cell to these signals lead to type II diabetes mellitus. Since previous studies have shown that changes in β-cell membrane potential and intracellular Ca^{2+} are crucial steps in regulated insulin secretion, our particular focus is on the modulation of β-cell electrical activity and intracellular Ca^{2+} levels by nutrient and neurohormonal signals. Specific projects include: 1) Investigation of the signaling pathways involved in stimulation of insulin secretion by the neurotransmitter, acetylcholine, using molecular methods to identify acetylcholine receptor subtypes in β-cells and knock-out mice to determine the functional roles of these receptors. 2) Studies of the roles of intracellular Ca^{2+} stores and store-operated ionic currents in the regulation of insulin secretion, using fluorescent intracellular Ca^{2+} indicators and direct ionic current measurements from rodent and human β-cells 3) Studies of β-cell function and dysfunction in *psammomys obesus*, an animal model of dietary induced obesity and diabetes.

Dr. De-Maw Chuang received his BS from National Taiwan University, PhD in Molecular and Cellular Biology from State University of New York at Stony Brook and postdoctoral training at the Roche Institute of Molecular Biology. Dr. Chuang is Chief of Molecular Neurobiology at NIMH and joined USUHS in 2002 as an Adjunct Professor in the Department of Psychiatry. Dr. Chuang's laboratory studies molecular and cellular actions of mood-stabilizers and antidepressant drugs. His group is particularly interested in the neuroprotective mechanisms underlying lithium-induced protection against glutamate-induced excitotoxicity in cellular models and animal models of neurodegenerative disoders such as stroke and Huntington's disease. The group also investigates the molecular mechanisms underlying the pro-apoptotic role of glyceraldehydes-3-phosphate dehydrogenase (GAPDH), sometimes referred to as a housekeeping gene, and the involvement of this gene in the pathogenesis of certain forms of neurodegenerative disorders.

Awardees:
USUHS Translational Research Program in the Defense Brain / Spinal Cord Injury Program

Student Fellowships:

Josh Murtie (Molecular and Cell Biology Graduate Program)
Roles of PDGF and FGF2 During In Vivo Remyelination

Research Grants:

Dr. Denes Agoston (Anatomy, Physiology & Genetics)
Glia Differentiation in the Developing and Injured Brain

Dr. Juanita Anders (Anatomy, Physiology & Genetics)
Combined OEC and Light Therapies Repair Spinal Cord Injury

Dr. Suzanne Bausch (Pharmacology)
NMDA Glutamate Receptors in Epileptogenesis

Dr. Zygmunt Galdzicki (Anatomy, Physiology & Genetics)
Metabotropic Glutamate Receptors in Spinal Cord Injury

Dr. Sharon Juliano (Anatomy, Physiology & Genetics)
Neurotrophins Restore Function After Forebrain Injury

Dr. Ann Marini (Neurology)
N-methyl-D-aspartate Receptor Neuroprotection in Hippocampal Neurons

Dr. Aviva Symes (Pharmacology)
TGF-β Signaling in Glial Scar Formation After CNS Injury

Dr. Ajay Verma (Neurology)
Metabolic Support for the Injured Nervous System

Dr. Guoqiang Xing (Psychology)
Neuroprotection by Yunnan Bai Yao in Brain Trauma

Dr. Xiang Yao (Anatomy, Physiology & Genetics)
The Role of the Ubiquitin-Proteosome System in TBI

USUHS Neuroscience Student Graduates

Spc. Anthony J. Williams completed his Ph.D. in Neuroscience on February 19 2003, under the direction of LTC Geoffrey Ling, Professor and Vice-Chairman of Neurology and Dr. Frank Tortella of Walter Reed Army Institute of Research. Tony worked full-time as an Army specialist while completing his degree and was recently featured in articles in the Frederick News Post (Mar 4, 2003) and WRAIR News and Events (http://wrair-www.army.mil/News&Events/News/DrWilliams.htm).

NEUROPROTECTION PROFILE OF THE HIGH AFFINITY NMDA RECEPTOR ANTAGONIST CONANTOKIN-G

Anthony J. Williams

Conantokin-G (Con-G or CGX-1007), a potent NR2B subunit selective NMDA receptor antagonist, was evaluated for its neuroprotective properties in experimental models of neuronal injury. In primary neuronal cultures Con-G was shown to decrease excitotoxic calcium responses to NMDA and provide 100% neuroprotection against hypoxia/hypoglycemia (34 μM[13-91]), NMDA (77 μM[42-141]), glutamate (819 μM[346-1937]) or veratradine (2136 μM[1508-3026]) induced injury (numbers in parentheses indicate EC_{50} and 95% confidence limits). Con-G (0.1-1 μM) also provided up to 80% protection against staurosporine-induced apoptotic injury ($P<0.01$, n = 12/group), which was linked to the NR2B subunit. For *in vivo* brain injury studies, middle cerebral artery occlusion (MCAo) in the rat was used as a model of transient focal brain ischemia. In this model Con-G (0.01-2.0 nmoles, i.c.v.) reduced brain infarction and improved both neurological and electroencephalographic (EEG) recovery as evaluated both 24 and 72 h post-injury. The maximal neuroprotective effect was measured with the highest dose of Con-G tested (2.0 nmol, i.c.v) with an 89% reduction of core infarct volume ($P<0.05$, n = 6-10/group). Post-injury time course experiments demonstrated a therapeutic window out to at least 4 h from the start of the injury. These neuroprotective effects were also associated with a 50% reduction in the early expression (i.e. 1-4 h) of the *c-fos* gene ($P<0.05$, n = 3-4/group), a preservation of Bcl-2 immunoreactivity at 24 h ($P<0.05$, n = 4), and with a reduction in DNA strand breaks in the ischemic hemisphere as evaluated 24 h post-injury ($P<0.05$, n = 6/group). Clinically relevant routes of administration were evaluated by administering intrathecal (i.t.) injections of Con-G (20-160 nmol), which provided up to 62% reduction in brain infarction ($P<0.05$, n = 8-9/group) along with significant neurological recovery and a therapeutic window of up to 8 h post-injury. Con-G (i.t.) treatment was also associated with fewer ischemia-induced EEG seizures with a positive albeit non-significant trend ($P>0.05$, n = 6-7/group) between the number of brain seizures and infarct volume. Intrathecal Con-G was not associated with changes in *c-fos* gene expression although, similar to i.c.v. administration, Bcl-2 immunoreactivity was preserved in cortical tissues ($P<0.05$, n = 3/group) and presence of TUNEL positive cells decreased at 24 h ($P<0.05$, n = 6/group). These data provide evidence for the potent and highly efficacious effect of Con-G as a neuroprotective agent with an excellent therapeutic window for the potential intervention against ischemic/excitotoxic brain injury in humans.

USUHS Neuroscience Student Graduates

Maj. Thomas E. Ceremuga completed his Ph.D. in Neuroscience on March 13, 2003 under the direction of Dr. Joseph T. McCabe, Professor and Vice-Chair of Department of Anatomy, Physiology & Genetics. Tom is planning to return to duty in the Army Nursing Corp.

CULLIN 5 EXPRESSION IN THE RAT: CELLULAR AND TISSUE DISTRIBUTION, AND CHANGES IN RESPONSE TO WATER DEPRIVATION AND HEMORRHAGIC SHOCK

Thomas E. Ceremuga

Protein degradation by ubiquitination and the 26S proteasome is used to modulate the steady-state levels of proteins and to regulate cellular processes. Proteins become targets of the proteasome by covalent attachment of polyubiquitin chains, which requires three main enzymes (E1, E2, and E3). It is the E3 ubiquitin ligases that control the selection and specificity of substrate ubiquitination. Cullin-5 (Cul-5), a member of the cullin family of E3 ubiquitin ligases, remains obscure. The goals of this research project were to characterize Cul-5, and investigate its response to cellular stresses of water deprivation and hemorrhagic shock in the rat.

Northern blotting of poly(A)$^+$ RNA from various rat tissues demonstrated the cul-5 transcript is approximately 6.3 kb. Reverse transcription-polymerase chain reaction (RT-PCR) indicated cul-5 mRNA is present in twelve tissues examined: brainstem, cerebral cortex, cerebellum, hypothalamus, aorta, gastrointestinal tract, heart, kidney medulla, liver, lung, skeletal muscle, and spleen. Quantitative realtime PCR confirmed RT-PCR results that Cul-5 mRNA is ubiquitously expressed and that levels are similar in all tissues. Cellular specificity examination showed cul-5 mRNA expression in rodent neuronal, glial, and vascular endothelial cells in the central nervous system (CNS) via RT-PCR. We corroborated these data by immunocytochemical techniques demonstrating Cul-5 protein presence in neurons, astrocytes, blood vessels, and choroid plexus in rat.

Functional assays measured cul-5 mRNA expression responses to water deprivation and hemorrhagic shock. Quantitative realtime PCR showed significant cul-5 mRNA elevations in the rat cerebral cortex (3 fold, $p<0.001$), hypothalamus (2 fold, $p<0.007$), and kidney (1.5 fold, $p<0.04$) following 48 hours of water deprivation. Water deprivation for 24 hours or rehydration (24 hours access to water following 48 hours of water deprivation) also increased kidney cul-5 mRNA levels (1.5 fold, $p<0.04$ and 3 fold, $p<0.001$ respectively). Hemorrhagic shock was used as a second *in vivo* cellular stress model. Rats were subjected to volume controlled (27 ml/kg) hemorrhage over 10 minutes and kept in shock for 60 minutes. Levels of cul-5 mRNA were significantly increased in the brainstem and cerebellum (1.6 fold, $p<0.01$ and 1.5 fold, $p<0.05$ respectively), and decreased in the hypothalamus (0.5 fold, $p<0.05$) compared to sham-treated rats.

We determined that Cul-5 is synthesized in all tissues and organs we examined, and in neurons, glia, and endothelial cells in the CNS. Using two paradigms of cellular stress, we found cul-5 mRNA levels in the CNS are altered by water deprivation and by hemorrhagic shock. However, much remains to be revealed concerning what precise physiological role(s) Cullin-5 plays in various cellular processes.

Combined: Monthly Lunch Group for Graduate Students & Student-run Neuroscience Journal Club

The monthly lunch group for USUHS Neuroscience graduate students (NSL) is sponsored by the Neuroscience Program. Its goals are to discuss issues important to graduate students and to facilitate peer support . The Neuroscience Journal Club (JC) aims to keep students abreast of current literature, offers an opportunity to discuss Neuroscience topics and provides an informal setting to practice presenting research. The combination NSL/JC Meetings will be held the 1st Tuesday of each month from 12pm-1pm for Nov-May. Meetings will be extended to 11:30am-1pm for June-Oct to allow ample time for both the JC and NSL. These are informal, student run meetings designed to help graduate students get through their graduate careers. If you have suggestions for a meeting or journal club topic, please contact Alisa Schaefer (aschaefer@usuhs.mil) or Tara Romanczyk (tromanczyk@usuhs.mil). JC presenters to be determined.

2003/2004 SCHEDULE (Topics and dates subject to change)

April:	*NSL topic:* The Qualifying Exam
May:	*NSL topic:* How to Read a Research Paper
June:	*JC / NSL topic:* Grad School Burnout
July:	*JC only*
August:	*JC only*
September:	*JC / NSL topic:* Orientation to Grad School: Finding a Lab, Choosing a Mentor, Rotations
October:	*JC / NSL topic:* Attending Conferences and Poster Presentations
November:	*NSL topic:* Finances
December:	Holiday Luncheon
January:	*NSL topic:* Applying for Grants & Fellowships
Febuary:	*NSL topic:* Thesis Writing
March:	*NSL topic:* Oral Presentations

Your Graduate Student Representative

is currently Tara Romanczyk . Each USUHS graduate program has a Student Program Representative whose role is to serve as liaison between administration/faculty and the graduate students through the dissemination of pertinent information. This position also allows students to raise concerns and issues that can then be addressed through more formal channels. If you have any questions, comments or concerns, please contact Tara Romanczyk.

2002/2003 Neuroscience Program Executive Committee

Regina Armstrong, Ph.D.	**Program Director, Anatomy, Physiology & Genetics**	**301-295-3205**
He Li, Ph.D.	**Psychiatry**	**301-295-3295**
Aviva Symes, Ph.D.	**Pharmacology**	**301-295-3234**
Sharon Juliano, Ph.D.	**Anatomy, Physiology & Genetics**	**301-295-3673**
Leslie McKinney, Ph.D.	**Anesthesiology**	**301-295-3021**

Newsletter Editor

Suzanne Bausch, Ph.D.	**Pharmacology**	**301-295-3226**